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Comparative toxicity of silver nanoparticle and ionic silver in juvenile common carp (*Cyprinus carpio*): Accumulation, physiology and histopathology

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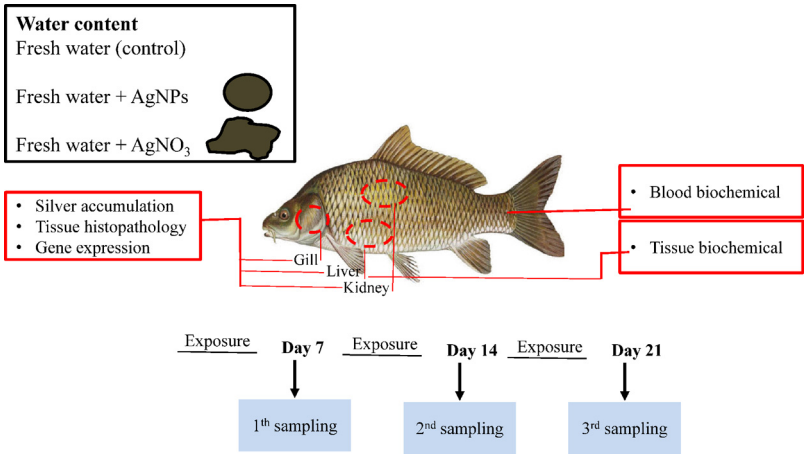
GRAPHICAL ABSTRACT



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ABSTRACT

Many studies have investigated the potential negative effects of silver on aquatic organisms, but most focused on short-term exposure in few species. Moreover, there are many uncertainties about differences in potential toxicity mechanisms and adverse effects of silver nanoparticles (AgNPs) and ionic form of silver ($AgNO_3$). We investigated chronic effects of AgNPs and $AgNO_3$ on the juvenile common carp (*Cyprinus carpio*). AgNPs and $AgNO_3$ accumulated in the liver, gill and intestine, respectively and highest was related to AgNPs. Our results indicated, silver uptake was accompanied with histological alteration in the target organs such that different tissue lesions were observed in exposed groups. Superoxide dismutase (SOD), catalase (CAT) and lactate dehydrogenase (LDH) activity and also hsp70, ghrelin and IGF-1 genes expression were induced in both forms. After 7 days, highest hsp70 gene expression was observed in $AgNO_3$ treatment and highest ghrelin and IGF-1 gene expression was observed in AgNPs treatment. The results revealed that adverse effects of AgNPs on different aspects of the health of juvenile common carp, may not be solely a result of particle dissolution. In addition, the main toxic mechanism of AgNPs was probably related to the accumulation of silver followed by the molecular and oxidative stress response.

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1. Introduction

Silver nanoparticles (AgNPs) are one of the most used nanomaterials and due to their unique properties, they have been used in many consumer products like, textiles, cosmetics, plastic, food packaging, children's toys and medical appliances [1,2]. The widespread applications of AgNPs have led to their release into the environment, resulting in growing concern of potential risk [1]. Aquatic environments are generally considered as ultimate destination for these NPs [3]. According to a modelling study, about 60 tons of silver enter the surface waters anually [4]. In other studies, the concentration of these NPs in freshwater environment was predicted at 0.01–100 ng L^{-1} [5,6]. Nevertheless, it can be said that due to inevitable increase in consumption of this product, the amount of AgNPs in the aquatic ecosystems will increase in the near future and this will lead to unknown hazards in aquatic organisms.

Many studies have been carried out on the acute toxicity of NPs to aquatic organisms [7,8], whereas long-term exposures to NPs were necessary for risk assessment of NPs in aquatic animals [9]. Few information are available on the effects of NPs on growth related genes such as ghrelin and IGF. This raises an interesting question about the effect of metallic NPs on growth related genes in aquatics. Proper growth reflects the favorable conditions in external and internal organs of a living organism [10]. Despite its importance, there are many uncertainties about expression mechanism of growth related genes in response to NPs. Insulin-like growth factor 1 (IGF-I) is a key molecule that regulates many physiological functions including survival, reproduction and migration [10]. Ghrelin in another key molecule that regulates physiological functions that are related to growth and food intake [11]. In contrast to IGF-I that are expressed in many organs such as gill, liver and kidney, ghrelin is mainly expressed in the fish gut [11]. Exposure of aquatic organisms to different environmental stressors leads to the synthesis of conserved proteins named heat shock proteins (hsp). Hsp70 is well characterized among others, and is used as general stress response for monitoring environmental stressors [12]. Also in some studies, organ specific expression of hsp70 was reported [13].

In previous studies, it was found that after exposure of fish to different engineered NPs, these materials accumulate in different tissues [9,14]. Bioaccumulation is achieved via different routes, but crossing over the gill and skin epithelium are likely to be the major routes [14]. There is limited data on AgNPs bioavailability, distribution and accumulation after exposure of fish. Bioaccumulation of metallic NPs, can be associated with oxidative stress and histological alteration [9,15]. In addition, pathology is a simple and conventional method for evaluating negative effects of chemicals on structure of different tissues [16]. Histopathology shows initial signs of lesions and alterations, so, evaluation of histopathological alteration in response to NPs can be used as appropriate tool to assess aquatic health.

Different factors can affect the toxicity of metallic NPs such as nanoscale size, high surface area, shape, structure and free ions [17]. Free ions are one of the important factors that affect AgNPs toxicity and different factors such as salinity, hardness and pH cause the release of Ag^+ [18]. Free ion of silver is very toxic and its toxicity is probably related to the strong affinity to thiol groups and also structural similarity with some ions such as sodium, thus Ag^+ can disturb normal physiological behaviors [6].

Against this background, the aim of this study was to carry out a comprehensive analysis of the effects of two chronic concentrations of AgNPs in juvenile common carp. To this end, a wide range of endpoints were used to evaluate the toxicokinetics, antioxidant response, molecular response and tissue pathology. Moreover, to explore the differences in the toxicological effects of nanosilver and soluble silver, simultaneously, another experiment was performed to compare the chronic effects of two forms of silver. The results of this study indicate the effects of chronic concentrations of AgNPs on different aspects of the health of juvenile common carp, and also demonstrate the difference in the toxicity of AgNPs against $AgNO_3$.

2. Materials and methods

2.1. Silver nanoparticles (AgNPs) and silver nitrate ($AgNO_3$)

Commercially colloidal AgNPs were purchased from Nano Nasb Pars Co. (Nanocid; Tehran, Iran). According to the manufacturer's information, there were 4000 mg L^{-1} of silver in AgNPs solution. Average particles size was 20 nm and particles had spherical morphology and purity of > 99%. $AgNO_3$ pellets with a purity of > 99.5% were prepared from Sigma- Aldrich (Steinheim, Germany). The stock suspensions were prepared by adding AgNPs and $AgNO_3$ pellets into distilled water and then added to exposure tanks.

2.2. AgNPs characterization

Diameter of AgNPs in stock solution was characterized with Transmission Electron Microscope (TEM; Hitachi, Japan). Hydrodynamic diameter and zeta potential of AgNPs in solution was measured by Dynamic Light Scattering (DLS; Zetasizer, Malvern Instruments). Briefly, at 0 and 24 h of experiment, appropriate volume of AgNPs solution was applied to cuvette and assessed with DLS.

To determine dissolution rate in each culture condition, Amicon Ultra Centrifugal Filters (3 kDa, Millipore, Germany) were used. Briefly, after 24 h from dosing, 5 ml of AgNPs solution was sampled and added to device, then centrifuged at 8000 rpm for 50 min. Then, flow through was collected and Ag^+ were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). To have reliable toxicity data, working concentration is essential, therefore in this study after 24 h from dosing, concentrations of silver in the exposure tanks were measured with ICP-MS.

2.3. Fish

Juvenile common carp (length: 16.3 ± 1.9 cm, weight: 9.78 ± 1.92 g) were purchased from a local commercial hatchery. For acclimatization purposes, the fish were kept in 1000 L tank for two weeks under 14 h light: 10 h dark photoperiod cycle. In all treatments, tanks were aerated continuously and some physiochemical properties of water such as dissolve oxygen, water temperature and pH were measured daily and maintained at 7.8 mg L^{-1}, 21.1 °C and 7.3, respectively. Fish were fed with commercial extruder diet (Mazandaran Animal & Aquatic Feed Com. Sari, Iran) and 50% of water was exchanged daily to remove metabolites and unused food.

2.4. Determination of median lethal concentration (LC_{50})

Acute toxicity study was carried out according to Organization for Economic Cooperation and Development (OECD) Guideline 203 (OECD, 1992), under static test condition. For each form of silver, 6 concentrations were selected. Nominal concentrations for both forms of silver were 0, 0.1, 0.125, 0.187, 0.25 and 0.5 mg L^{-1}. The fish were not fed for 24 h before the start of experiment. Juvenile common carp were randomly assigned to 100 L tanks and 3 replicates were considered for each concentration. Fish mortality was recorded every 24 h up to 96 h and then dead fish were removed. LC_{50} was measured using Probit analysis method.